First Structure of a Key Molecular Engine

HisP Protein Structure Carries Important Medical Implications

ATP-binding cassette (ABC) transporters (also known as traffic ATPases) form a large family of proteins responsible for movement of biochemical compounds through cell membranes in both prokaryotes and eukaryotes. Researchers from Berkeley Lab and the University of California, Berkeley, have used the Macromolecular Crystallography Facility at the Advanced Light Source (ALS) to obtain the crystal structure of the ATP-binding subunit HisP of histidine permease, an ABC transporter from *Salmonella typhimurium*. The structure, obtained with a resolution of 1.5-Å, provides a basis for understanding properties of both normal and defective ABC transporters, such as the cystic fibrosis transmembrane conductance regulator (CFTR) that is involved in cystic fibrosis.

ABC transporters variously act as pumps that propel substances through membranes, channels that allow their passage, and regulators that control other membrane proteins. Their general structural features, known from biochemical studies, include membrane-spanning domains between which the transported substances pass and nucleotide-binding domains, which are the molecular engines that drive the pumps or open and close the channels using the energy released by the binding and hydrolysis of ATP. Owing to the substantial similarity of the nucleotide-binding domains in both prokaryotic and eukaryotic cells, structural details found in one organism are expected to apply to others.

In *S. typhimurium*, the ABC transporter histidine permease contains two identical nucleotide-binding domains, known as HisP, that together form a dimer. Using multiple-wavelength anomalous diffraction (MAD) methods to obtain phase information about the diffracted beam, the Berkeley group has solved the structure of HisP with a resolution of 1.5 Å. A four-wavelength MAD data set was obtained in just two hours at the ALS from a cryo-cooled selenomethionine HisP crystal. The structure of the HisP monomer exhibits an unusual L shape with two thick arms, one of which holds the ATP binding site and the other of which is proposed to be in contact with a membrane-spanning domain.

The structure determined by the Berkeley researchers is consistent with a large body of biochemical, genetic, and biophysical data obtained from isolated HisP and HisP in histidine permease. This consistency suggests that the structure of the crystal is functionally equivalent to HisP in cells. There are numerous questions remaining for future work. For example, it appears that the binding and hydrolysis of ATP in one arm result in conformational changes in the other arm that are passed on to a membrane-spanning domain, but the mechanism is not yet known in any detail, The purpose of the dimer structure resulting in two molecular engines is also not yet known.

ABC transporters are increasingly recognized as the causes of genetic diseases in humans. For example, mutations in the gene that codes for CFTR cause cystic fibrosis, a lethal disease that occurs in about 1 in 3300 live births in the United States and Canada. Although histidine permease is a pump in a prokaryote and CFTR is a channel in a eukaryote, the nucleotide-binding domains in each are homologous. The Berkeley researchers used their HisP findings to propose a structural basis for the consequences of the known mutations of CFTR. The most common mutation, found in 90% of all cystic-fibrosis patients, leads to incorrect protein folding and transit. Other mutations may inhibit ATP binding or hydrolysis, thereby turning off the engine, or disrupt the interaction with the membrane-spanning domains, thereby putting the engine in neutral. This kind of information may eventually lead to a treatment for cystic fibrosis. Moreover, the ability to correlate the properties of CFTR mutants with the crystal structure of HisP indicates that HisP is a good model for the nucleotide-binding domains of ABC transporters in general.

Sung-Hou Kim (510-486-4333), Physical BioSciences Division, E. O. Lawrence Berkeley National Laboratory and Department of Chemistry, University of California, Berkeley.

L.-W. Hung, I. X. Wang, K. Nikaido, P.-Q. Liu, G. F.-L. Ames, and S.-H. Kim, "Crystal structure of the ATP-binding subunit of an ABC transporter," *Nature*, **396** (1998) 703.

RESEARCH FUNDING: Office of Biological and Environmental Research, U. S. Department of Energy; National Institutes of Health. Operation of the ALS is supported by the Division of Materials Sciences, U. S. Department of Energy.

FIRST STRUCTURE OF A KEY MOLECULAR ENGINE

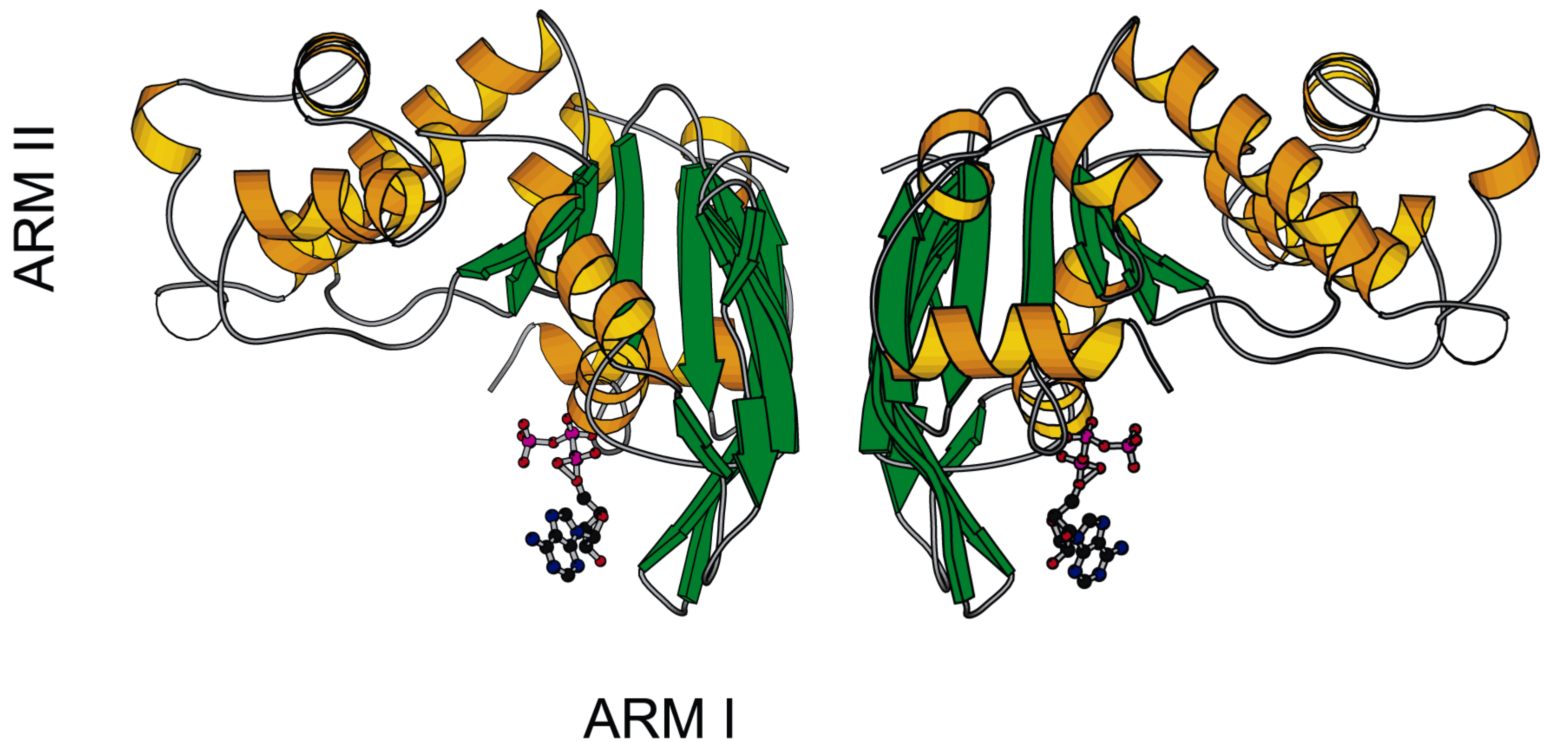
HisP Protein Structure Carries Important Medical Implications

- **ABC transporters are common proteins in cells**
  - *Molecular engines for movement of compounds through cell membranes*
  - *Many are of medical significance*
- **Histidine permease in *Salmonella typhimurium***
  - *Molecular pump consisting of four domains*
  - *Two identical nucleotide-binding domains (HisP) drive the pump*
- **HisP structure obtained at Macromolecular Crystallography Facility**
  - *1.5-Å resolution using MAD phasing*
  - *Unusual L-shape with thick arms and an ATP-binding site*
  - *Structure correlated with biochemical, genetic, and biophysical properties*
- **Implications for cystic fibrosis**
  - *Cystic fibrosis caused by mutations in gene for CFTR, an ABC transporter*
  - *Nucleotide-binding domains in CFTR homologous to HisP*
  - *HisP suggests a structural basis for CFTR defects*

L.-W. Hung (Berkeley Lab); I. X. Wang, K. Nikaido, P.-Q. Liu, and G. F.-L. Ames (University of California, Berkeley); and S.-H. Kim (University of California, Berkeley, and Berkeley Lab.). [*Nature* 396 (1998) 703]

FIRST STRUCTURE OF A KEY MOLECULAR ENGINE

HisP Protein Structure Carries Important Medical Implications

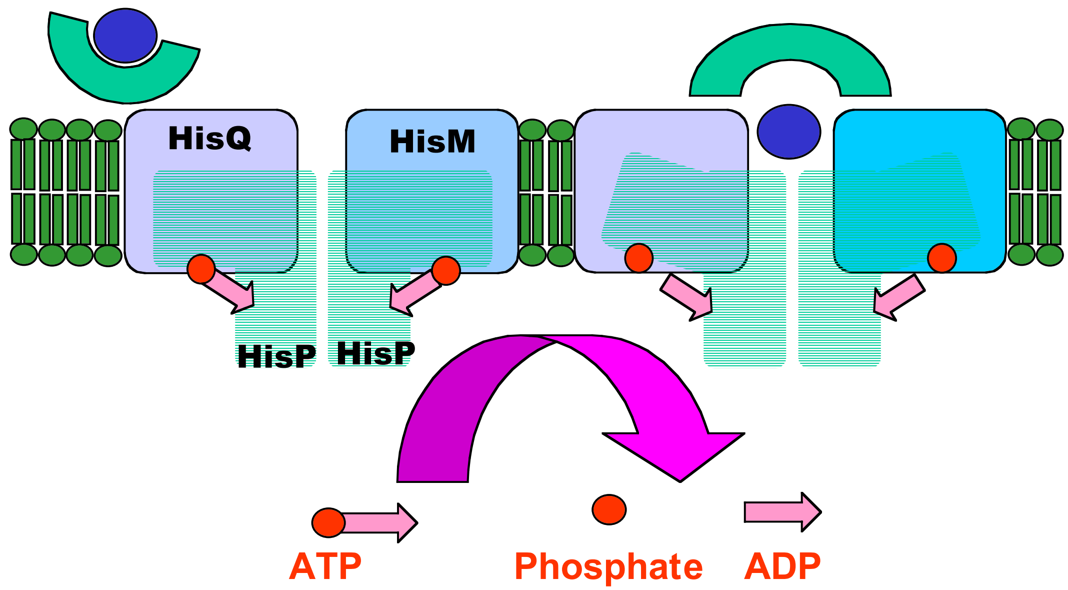


Structure of HisP exhibits two arms in an L-shaped configuration. ATP (shown in ball-and-stick representation) resides in a binding pocket toward the bottom of Arm I. Two HisP units together form a dimer.

L.-W. Hung (Berkeley Lab); I. X. Wang, K. Nikaido, P.-Q. Liu, and G. F.-L. Ames (University of California, Berkeley); and S.-H. Kim (University of California, Berkeley, and Berkeley Lab.). [*Nature* 396 (1998) 703]

FIRST STRUCTURE OF A KEY MOLECULAR ENGINE

HisP Protein Structure Carries Important Medical Implications



Left – Histidine permease molecular pump in a cell membrane (green) comprises two membrane-spanning domains (HisQ and HisM) and two identical nucleotide-binding domains (HisP).

Right – Operation of the pump is driven by binding of ATP and hydrolysis to ADP, which are proposed to cause conformational changes in HisP that are transmitted to HisQ and HisP.

L.-W. Hung (Berkeley Lab); I. X. Wang, K. Nikaido, P.-Q. Liu, and G. F.-L. Ames (University of California, Berkeley); and S.-H. Kim (University of California, Berkeley, and Berkeley Lab.). [*Nature* **396** (1998) 703]